APBDRF

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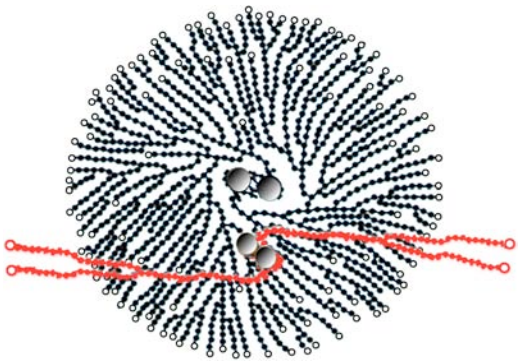

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PRESENTATIONS

Deep Intronic GBE1 Mutation in Manifesting Heterozygous Patients with Adult Polyglucosan Body Disease

Orhan Akman
Columbia University Medical Center, New York, New York

Adult Polyglucosan Body Disease (APBD) is a severe, late-onset autosomal recessive disorder affecting the central and peripheral nervous systems. It generally progresses after 4 or 5 decades of life. Its main symptoms are variable combinations of peripheral neuropathy, myelopathy, neurogenic bladder and cognitive impairment. APBD is caused by mutations in a gene encoding glycogen branching enzyme (*GBE1*). Most patients are of Ashkenazi Jewish descent; 70% of these patients have bi-allelic p.Y329S mutation. The remaining 30% are heterozygous for the p.Y329S mutation. A second mutation had not been found until now, despite whole-genome sequencing. This had raised the possibility that p.Y329S heterozygous cases were somehow 'manifesting heterozygotes.' We have now resolved this question and identified a deep-intronic mutation,

GBE1-IVS15+5289_5297delGTGTGGTGGinsTGTTTTTTACATGACAGGT,

which acts as a gene-trap and creates a pseudo last-exon. Although mRNA is stable, the resulting enzyme is degraded. This second most common APBD mutation now explains all Ashkenazi Jewish cases.

Guaiacol Treatment Decreases Resting Glycogen Synthase Activity and Expands the Life Span in the Mouse Models of APBD

Orhan Akman

Guaiacol is a naturally occurring organic compound with the formula $C_6H_4(OH)(OCH_3)$. It is synthesized naturally by a variety of organisms. Guaiacol contributes to the flavor of many food and organic materials, such as roasted coffee and smoke. Our preliminary experiments have shown that Guaiacol decreases glycogen content in mouse embryonic fibroblasts in a dose-dependent manner by 70%. Similarly, at two doses tested (20 and 45 mg/kg), mice have shown low glycogen content in the liver, although muscle and brain glycogen concentrations remained high. However, treatments expand the life span in mice with PGK-Neomycin cassette from 7 months to 10 months, and, in mice with point mutation only, from 15 months to 20 months. Guaiacol has decreased the glycogen synthase activity but did not prevent the allosteric activation by glucose 6 phosphate. Western blot analyses have shown hyper phosphorylation of glycogen synthase in liver and muscle which explains lower steady state activity. We are currently analyzing the side effects of Guaiacol in liver and kidneys. Although the glycogen content in muscle and brain remained unchanged, the positive effect of Guaiacol in life span is remarkable. Therefore, Guaiacol should be considered as an alternative treatment.

Effect of TGMs on GBE1 Activity and Binding to Membranes: Implications in APBD Therapy

Rafael Álvarez and **Pablo V. Escribá**
University of the Balearic Islands, Palma, Spain

Adult Polyglucosan Body Disease (APBD) is an adult onset progressive neurodegenerative disorder associated with Y329S homozygous mutation in the *GBE1* gene, which causes a reduction in activity in 1,4-alpha-glucan-branching enzyme (GBE1). Both the wild type and Y329S forms were cloned in the pFastBac plasmid, expressed in Sf9 cells and purified by affinity chromatography. The activity and binding of these proteins to model membranes (liposomes) was investigated in the presence and absence of synthetic tryacilglyceromimetics (TGMs). It was found that the mutated GBE1 protein had a higher binding to membranes than the wild type protein. This result suggests a possible mis-folding of Y329S that was further confirmed by thermal stability experiments. Because the cytoplasm is full of membranous systems, it is feasible that the Y329S form could associate to them in a way that could reduce its activity. Currently, triheptanoin (TH, a triacylglycerol) is in clinical trials (ClinicalTrials.gov identifier NCT00947960) to investigate its efficacy against APBD. Interestingly, TH induced changes in the binding of GBE1 to membranes and increased the activity of Y329S. In this context, TGMs have a slower metabolic rate than triacylglycerols and could constitute a better therapeutic tool than TH. Indeed, some TGMs, such as TGM0 and TGM5, induced relevant increases in the activity of APBD in the presence of membranes. Although further efficacy studies in cell and animal models of APBD are needed, these TGMs constitute a potential therapeutic tool for the treatment of this condition.

Targeted and Non-Targeted Approaches for Treating Adult Polyglucosan Body Disease

Or Kakhlon
Hadassah Hebrew University of Jerusalem, Israel

Adult Polyglucosan Body Disease (APBD) is a rare, as yet incurable neurological disorder caused by accumulation of poorly branched, non-soluble polysaccharide aggregates called polyglucosan bodies (PB). Obstruction of brain cells by PB in APBD leads to progressive neurodegeneration and ALS-like symptoms. Polyglucosans form by excessive, glycogen synthase (GS)-mediated, glucan elongation, at the expense of its glycogen branching enzyme (GBE)-mediated branching. Therefore, to find a cure for APBD, I have been collaborating with several researchers to develop and test *non-targeted* approaches focused only on PB elimination as well as *targeted* approaches for stabilizing GBE:

1. *Non-targeted* high throughput screenings (HTS) have discovered 16 new non-toxic candidates which reproducibly reduce PB level in patient-derived skin fibroblasts. These positive hits are being computationally clustered into pharmacophores and form the basis for rational design of pharmacokinetically superior compounds. In addition, docking of discovered hits into the putative targets of GS, protein targeting to glycogen, glycogenin, and GBE is being conducted. *En route* to personalized medicine, we aim to test the effects of hits on numerous cellular phenotypes in cells derived from several patients.

2. *Targeting GBE*: I have collaborated with Drs. Yue and Michaeli to discover a peptide which stabilized and biochemically activated GBE Y329S, presumably by interacting with the cavity generated by the mutation on mutant GBE surface. We aim to use this technology to design more peptides for the targeted stabilization of the Y329S and other GBE mutations, allowing us to potentially treat a broader spectrum of APBD patients.

3. Preliminary results from Dr. Escribá indicate that GBE Y329S reversible interaction with membranes regulates its activity. Moreover, Dr. Escribá's results demonstrate that some triacylglyceride mimetic (TGM) molecules increase purified GBE Y329S activity up to two-fold. In

the second approach targeting GBE, these TGM are currently being assessed in APBD patient-derived lymphocytes as the next step in their drug development. The ultimate goals are to design and synthesize more TGM molecules and eventually investigate the efficacy and safety of the best compounds in our APBD mouse models. Ultimately we aim that all three approaches will provide successful PB lowering and/or GBE stabilizing compounds which, through medicinal chemistry and trials, will become effective drugs for treating APBD in patients.

Carrier Frequency of Founder Mutation and Intronic Mutation

Ruth Kornreich
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The carrier frequency of the Ashkenazi Jewish GBE1 founder mutation, p.Y329S, has not been clearly established. We screened 2236 individuals who were self-reported to be 100% Ashkenazi Jewish and found 2209 individuals were wild type for the mutation and 27 were heterozygous for the mutation. The carrier frequency, therefore, in this group was found to be 1/83; 95% CI 1/123 – 1/56. Screening of approximately 2000 Ashkenazi individuals to determine the carrier frequency for the recently found deep intronic mutation, IVS15+5289_5297delGTGTGGTGGinsTGTTTTTTACATGACAGGT, will be presented.

Development of a Small Molecule Therapy for APBD and Degrading of Pre-Existing Polyglucosan Bodies

Erin Chown, Felix Nitshke, Peixiang Wang, Bart Pederson, Roman Melnyk, Orhan Akman, **Berge Minassian**
The Hospital for Sick Children, Toronto, Canada

Adult Polyglucosan Body Disease (APBD) is caused by autosomal recessive mutations in the glycogen branching enzyme (GBE1) gene and is characterized by the presence of malformed glycogen particles, called polyglucosan bodies. Polyglucosan bodies differ from glycogen particles in that they contain longer linear chains of glucose molecules and have a reduced frequency of branched chains, due to the diminished activity of GBE1. This altered structure causes the polyglucosan bodies to precipitate from solution and to impair cellular processes, most significantly in neurons. Therapeutic approaches for APBD must therefore involve prevention of polyglucosan body formation and degradation of pre-existing polyglucosan bodies. Towards preventing the formation of polyglucosan bodies, we hypothesized that reducing the elongation of linear glycogen chains could mitigate the reduced branch frequency in APBD. We therefore selected the chain elongation enzyme, glycogen synthase (GYS1), and its activator, protein targeting to glycogen (PTG), as potential therapeutic targets. We are crossing mouse models deficient in GYS1 (*Gys1+/-*) and PTG (*Ptg-/-*) with the APBD mouse model containing the Y329S GBE1 mutation (*Gbe1ys/ys*). We are characterizing these mice using a suite of behavioral, biochemical and histological analyses to determine whether knockdown of GYS1 or PTG rescues the murine APBD disease phenotype. We will report our progress. We will also report our progress on the development of a small molecule therapy for APBD and on degrading pre-existing polyglucosan bodies in the disease. Finally, we will provide update on our work with antisense oligonucleotides and CRISPRs against GYS1 and PTG.

A Randomized Controlled Treatment Trial of Triheptanoin in Patients with Adult Polyglucosan Body Disease

Mary Wallace and Rafael Schiffmann
Baylor Research Institute, Dallas, Texas

For the past 5 years Baylor Research Institute has conducted a treatment trial in APBD - protocol (IRB 009-103, FDA IND 105,246) approved 6/22/2009, clinicaltrials.gov identifier NCT00947960. Forty-two inquiries were received at the US site with 17 consenting between 10/26/2009 and 7/25/2014. The Paris, France site protocol was approved 2/2/2011 with accrual of 7 subjects. Twenty-four total subjects were accrued including both sites: 16 M, 8 F, age range 35-73 years at consent, mean age = 57 years. Nineteen of the 24 participants are of Ashkenazi Jewish descent. Nineteen subjects have completed the randomized controlled phase of the study, two are currently in the randomized phase of the study, and 3 dropped out prior to completion of the randomized controlled phase. Eighteen of the 19 subjects who completed the randomized phase continued in the open label phase of the study, with an average of 20 months on open label (range 2 to 50 months). Seven subjects are currently participating in the open label phase, and 2 are in the post triheptanoin discontinuation assessment. A total of 158 subject visits have been made to date. The study is now closed to new enrollment with unblinding, and data analysis is anticipated for summer 2015. In addition to testing a novel therapy, this study will provide a prospective quantitative natural history of APBD that is likely to be useful for future therapeutic clinical trials.

Correction of Glycogen Storage Disease Type IV by AAV-Mediated Gene Therapy

Haiqing Yi, Quan Zhang, Chunyu Yang, and **Baodong Sun**
Duke University Medical Center, Durham, North Carolina

***Background*:** Mutations in the glycogen branching enzyme (GBE1) gene cause glycogen storage disease type IV (GSD IV), resulting in deposition of less branched, poorly soluble polysaccharides (polyglucosan bodies, PB) in muscle, liver, and the central nervous system. There currently is no effective treatment for this disease. Recently a new mouse model of Adult Polyglucosan Body Disease (APBD) was developed by Drs. Craigen and Akman at Baylor College of Medicine (unpublished). The affected mice carry the Y329S mutation that leads to progressive accumulation of PB in all tissues.

***Objectives*:** To evaluate the efficacy of gene therapy with an AAV vector in GSD IV mice.

***Methods*:** Tissue histology was performed in GSD IV mice at age 10 days, 1, 3, and 6 months to evaluate disease progression. For gene therapy, an AAV vector containing human GBE1 cDNA driven by the ubiquitous CMV enhancer/chicken beta-actin (CB) promoter was packaged as AAV serotype 9 to achieve whole body gene transduction. The AAV vector ($5x10^{11}$ vector genome per mouse) was intravenously injected into 9-day-old GSD IV mice. Mice were uthanized at 3 months of age after overnight fasting and tissues were collected for biochemical and histological analyses.

***Results*:**

1) Histology revealed progressive disease progression in all tissues of the GSD IV mice. In liver, heavy PAS-positive glycogen was observed in all hepatocytes at young ages (10 days and 1 month), but became less with age. In skeletal muscles, glycogen deposition was undetectable at age of 1 month, but became readily visible at 3 months, and significantly increased at 6 months. In brain, scattered glycogen particles appeared at 3 months and significant glycogen loads were

observed in multiple types of cells. Heart seems less affected as glycogen was present in only very few myocytes even at 6 months.

2) In AAV-treated mice, GBE enzyme activity was highly elevated in heart (48-fold of wild-type) and skeletal muscles (2 to 3-fold of wild-type), and complete glycogen clearance was achieved in these tissues. Glycogen content was reduced significantly in the brain (by 51%), even though there was no significant increase in GBE activity. Neither GBE activity nor glycogen level was significantly affected in the liver by the AAV treatment.

***Conclusions*:** Systemic injection of AAV9-GBE vector at a young age completely corrected glycogen accumulation in the heart and skeletal muscles, and significantly reduced the glycogen level in the brain of GSD IV mice.

Structural Basis of Glycogen Branching Enzyme Deficiency and Pharmacological Chaperone Development

Wyatt Yue
Structural Genomics Consortium, University of Oxford, Oxford, UK

Glycogen branching enzyme 1 (GBE1) plays an essential role in glycogen biosynthesis, mutations of which lead to the heterogeneous early-onset glycogen storage disorder type IV (GSD IV) or the late-onset adult polyglucosan body disease (APBD). To better understand this essential enzyme, we crystallized human GBE1 in the apo form, and in complex with a tetra- or hepta-saccharide. The GBE1 structure reveals a conserved amylase core that houses the active center for the branching reaction, and harbors almost all GSDIV and APBD mutations. Expression of recombinant GBE1 p.Y329S resulted in drastically-reduced yield and destabilized protein compared to wild-type, suggesting this disease allele causes protein misfolding and may therefore be amenable to small molecule stabilization. Our data provides the starting point for the screening of small molecule chaperones, via a medium-throughput structure-guided approach. We are currently screening chemical compound libraries using a thermal stability assay. Hit compounds will then be assessed on the biochemical, structural and thermal properties of wild-type *vs.* mutant GBE1. The above studies will in the future facilitate *in vivo* assessment of hit compounds in patient-derived cells and animal models.

Antisense Therapy for Genetic Disorders

Tamar Grossman- *Isis Pharmaceuticals, Inc.*

Advances in deciphering the complex roles RNA plays in normal health and disease have been substantial over the past decade, and RNA is becoming an increasingly important target for therapeutic intervention. Antisense oligonucleotides (ASO) are perhaps the most direct therapeutic strategy to approach RNA, and ASO technology has emerged as a powerful alternative to conventional small molecule approaches or gene replacement strategies for the treatment of genetic disorders.

ASO are short, synthetic single-stranded DNA sequences designed to bind to target RNA by well-characterized Watson-Crick base pairing, and once bound to the target RNA, can modulate RNA function through a variety of post binding events. ASO-mediated gene silencing occurs either through degradative mechanisms, where the target RNA is cleaved by endogenous nucleases, or non-degradative mechanisms, where ASO binding sterically blocks or modulates translation, capping, or splicing. The majority of ASO drugs in development work through the RNaseH dependent degradation mechanism. For example, ASO inhibition of apolipoprotein B synthesis by KYNAMRO® (mipomersen sodium) is an effective therapy to reduce LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia. Furthermore, ASOs can utilize non-degradative mechanisms. For treatment of Spinal Muscular Atrophy (SMA), an ASO has been designed to correct SMN2 splicing and restore SMN expression. A phase 3 study with ISIS-$SMN_{Rx\ in}$ children with SMA was recently initiated.

This presentation will cover antisense technology platform and will include data from preclinical research and clinical trials with ASO drugs treating genomic disorders including new preliminary data with ASOs for treatment of APBD and Lafora disease.